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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, E. L. MARK, DIRECTOR. — No. 211.

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Presented by E. L. Mark, May 11, 1910. Received May 18, 1910.

The present investigation was made to ascertain the influence of inorganic and organic acids on the responses of the common manure worm, Allolobophora foetida (Sav.). Apart from its specific application, this problem bears upon the more general one of the relation of electrolytes — acids, bases, and salts — to physiological processes. For the suggestion of this problem and for much valuable advice as to method and interpretation, I am greatly indebted to Professor G. H. Parker.

The sense of taste even in man is not well understood. What its nature in an invertebrate may be, is only a matter of conjecture. Experiments designed to determine the relation between the chemical nature of solutions of acids to the sensations of taste in man, and especially to ascertain how closely the sensation corresponds to the degree of dissociation were made by Kahlenberg (**'98**) and by Richards (**'98**). It was my purpose to ascertain to what extent the conclusions reached in a study of human taste were applicable to a typical invertebrate like the earthworm.

The modern theories of solutions lead to the conclusion that the taste of a solution of an electrolyte must depend upon the taste of its ions, its undissociated molecules, or both; furthermore, the taste of a solution in which ionization is practically complete should be due simply to its ions. At dilutions of $\frac{N}{100}$ such as were used in the present investigation, strong acids, at least, would be almost completely dissociated. And their sour taste or stimulating value has been shown both by Kahlenberg and by Richards to depend upon hydrogen ion. It was further shown by Richards that the degree to which such acids stimulate is directly determined by the number of hydrogen ions.

For the purpose of the present study, three inorganic and one organic acid were used; they were hydrochloric, nitric, sulphuric, and

acetic acids. The degree of dissociation of these acids diminishes in the order named, hydrochloric acid being most and acetic acid least dissociated.

The strength of these solutions was so chosen that they should give distinct and measurable reactions, not sufficiently strong, however, to produce in any case lasting injurious effects upon the worm. Preliminary trial showed that an $\frac{N}{400}$ solution was serviceable for this purpose, since at higher concentrations the acids were so stimulating that the worms withdrew within two-tenths of a second, and, consequently, it was impossible to state whether at these concentrations one acid was a more effective stimulus than another.

The method of handling the worm was, with few modifications, like that used by Parker and Metcalf (:06) in their study of the reactions of earthworms to salts. The worms were thoroughly rinsed in tapwater till they were cleaned externally of foreign matter, and each worm was suspended by a silk thread passed through the posterior tip of its body and loosely tied. The worms thus prepared and numbered were kept singly in small, open, glass vessels lined with moistened filter paper, upon which the worms were allowed to crawl. The method of keeping them in tap-water was found undesirable because the water seemed to excite the secretions of mucus with the result that the mucus thus formed prevented the easy penetration of the acid to the skin of the worm and made the reaction-times slow and unreliable. The filter paper, on the other hand, served to remove all excess of mucus from the worms.

The apparatus with which the tests were made consisted of a base with an upright wooden post to which a pivoted arm was fixed. One of the ends of the arm was notched so that the silk thread carrying a worm could be inserted into it, and the other end was used as a handle by which the arm could be moved so as to raise the worm or lower it into the solution contained in a glass vessel on the base. When a test was to be made, a worm was taken up by its thread and attached to the arm of the apparatus. The superfluous water was drained from it, and, after it had lengthened fully, it was lowered gently, but quickly, into the solution to the depth of the anterior edge of the clitellum. As the tip of the worm cut the surface of the solution, a stop watch was started, and when as a result of the contraction of the worm, the tip withdrew from the solution, the watch was stopped. The interval of time thus recorded to fifths of a second was taken as the reaction-time for that particular experiment. If the worm failed to react after two minutes, the experiment was discontinued, and the worm was taken out of the solution. Such extremely long reactions were common

when the worms were kept in tap-water, but seldom occurred when they were allowed to crawl on the filter paper.

After the worm under ordinary circumstances had withdrawn from the solution, it was rinsed in tap-water, returned to its glass, and allowed to rest about five minutes before another trial with it was made. To rule out the possible disturbing factor of fatigue, it was decided to use no worm for more than a given, arbitrarily determined, number of reactions (twelve).

The acids were first experimented with in pairs; each acid being compared with every other one; hydrochloric acid, for instance, being compared with nitric, sulphuric, and acetic acids successively. Although it is not known that the particular sequence in which the acids are used makes any material difference, nevertheless to avoid any error which might possibly creep in by passing from a more to a less stimulating acid, the solutions were used alternately; for it is highly probable that when a strong solution is first applied, the effect of it is apt to be so vigorous as to obscure a subsequent response to weaker solutions. Where all the acids were compared at once the procedure adopted was to give each acid first place with at least one worm of those tested.

In tables I to XVI will be found the reaction-times of the earthworm, Allolobophora foetida, for the four different acids used.

Tables I to XVI. — Reaction Times, in Seconds, of Allolobophora foetida to Solutions of Nitric and Hydrochloric, Sulphuric and Hydrochloric, Sulphuric and Nitric, and Hydrochloric and Acetic Acids.

TABLE I.

$\frac{N}{400}$ HNO_3.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	0.4	25.0	38.2	1.2	12.6	32.4	18.3
II.	25.2	9.4	20.0	7.6	3.2	...	13.0
III.	18.8	11.2	29.8	11.0	10.8	...	16.3
IV.	0.2	0.6	0.8	0.4	0.4	7.2	1.6
V.	4.0	2.0	15.4	28.8	1.0	13.6	10.8
	General average						12.0

TABLE II.

$\frac{N}{400}$ HCl.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	0.6	5.0	8.0	17.6	22.8	17.8	11.9
II.	7.2	11.0	27.4	11.6	0.6	...	11.5
III.	20.0	5.4	14.0	12.0	2.4	...	10.7
IV.	0.4	0.6	0.4	0.4	0.4	1.0	0.5
V.	6.4	25.0	19.0	8.0	5.0	5.2	11.4
	General average						9.0

TABLE III.

$\frac{N}{400}$ HNO_3.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	0.4	14.4	14.0	1.0	1.0	1.0	5.1
II.	0.6	0.6	0.8	0.6	0.8	33.0	6.0
III.	0.4	24.0	7.0	20.0	21.0	11.2	13.9
IV.	0.2	0.2	0.6	0.6	0.6	35.2	6.2
V.	0.6	0.4	0.6	0.2	0.6	...	0.5
	General average						6.3

TABLE IV.

$\frac{N}{400}$ HCl.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	0.8	0.6	1.0	1.0	0.6	...	0.8
II.	0.8	0.8	0.8	1.0	1.0	1.2	0.9
III.	24.4	21.4	30.6	16.4	21.0	23.0	22.8
IV.	0.4	0.2	0.2	0.2	0.4	1.0	0.4
V.	0.4	0.6	0.6	0.8	...	...	0.5
	General average						5.0

TABLE V.

$\frac{N}{400}$ H_2SO_4.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	33.2	23.8	13.8	29.0	30.0	34.0	27.3
II.[1]	0.8	36.0	71.0	25.0	61.6	43.0	...
III.	0.8	1.2	14.0	1.0	22.4	20.8	10.0
IV.	0.2	0.6	1.0	0.6	1.0	2.0	0.9
V.	0.2	39.4	20.6	2.6	26.2	15.0	17.3
	General average						11.1

[1] Owing to the great variation in the readings obtained in Tables V and VI with worm number II, it was decided to discard these readings in making the general average.

TABLE VI.

$\frac{N}{400}$ HCl.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	26.2	0.2	1.2	31.0	40.0	51.6	25.0
II.[1]	54.0	62.8	65.2	34.0	0.4	31.0	...
III.	1.0	0.4	1.0	0.2	0.4	0.2	0.5
IV.	0.2	0.4	0.4	0.2	0.8	0.2	0.3
V.	1.0	0.2	0.4	1.0	0.6	16.0	3.2
	General average						5.8

TABLE VII.

$\frac{N}{400}$ H_2SO_4.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	7.0	30.8	0.6	22.4	26.4	8.2	15.9
II.	0.4	4.0	3.2	1.0	1.8	25.0	5.9
III.	0.4	0.6	0.4	1.0	2.0	8.4	2.1
IV.	25.4	0.6	22.8	5.0	0.4	0.8	9.1
V.	18.6	17.6	22.2	10.2	11.0	14.6	15.7
	General average						9.7

TABLE VIII.

$\frac{N}{400}$ HCl.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	14.6	0.4	0.6	8.4	19.0	17.0	10.0
II.	0.2	1.0	0.6	1.0	4.8	40.4 [1]	1.5
III.	0.4	0.4	0.2	0.4	0.8	2.2	0.7
IV.	0.4	22.8	15.0	2.4	23.2	22.0	14.3
V.	0.2	0.2	17.6	8.0	16.6	33.6 [1]	8.5
	General average						7.0

[1] The readings marked [1] show a great variation from the readings recorded in the other five tests. In the general average they were not included.

TABLE IX.

$\frac{N}{400}$ H_2SO_4.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	1.6	19.4	46.2	52.4	30.2	39.0	31.4
II.	21.0	8.2	20.0	16.2	8.0	32.0	17.5
III.	14.8	3.2	16.0	17.2	9.0	33.0	15.0
IV.	13.0	18.0	1.0	12.0	2.0	0.6	7.7
V.	15.2	18.2	16.2	0.2	0.2	...	10.0
	General average						16.4

TABLE X.

$\frac{N}{400}$ HNO_3.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	1.2	30.0	54.0	39.6	36.0	67.6	38.0
II.	19.2	22.0	31.0	19.2	13.0	40.0	24.0
III.	22.6	4.0	9.0	21.0	18.2	21.0	15.9
IV.	2.4	0.6	0.4	1.4	2.0	11.2	3.0
V.	0.2	17.0	11.4	0.4	0.4	...	5.9
	General average						17.3

TABLE XI.

$\frac{N}{400}$ H_2SO_4.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	17.0	12.0	8.2	11.6	33.8	11.6	15.7
II.	20.0	4.2	5.2	12.0	7.0	18.8	11.2
III.	19.0	15.4	12.0	0.6	1.0	...	9.6
IV.	12.0	27.4	48.2	20.4	11.0	47.0	27.6
V.	14.6	15.0	13.8	7.6	0.2	...	10.2
	General average						14.8

TABLE XII.

$\frac{N}{400}$ HNO_3.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	11.0	2.2	16.0	7.0	14.0	23.0	12.2
II.	20.8	7.0	1.2	5.2	11.0	2.0	7.8
III.	15.2	0.4	15.8	0.6	0.4	...	6.4
IV.	20.6	11.4	17.0	22.6	49.0	42.0	27.1
V.	19.4	0.8	15.0	11.0	4.6	...	10.1
	General average						12.7

TABLE XIII.

$\frac{N}{400}$ HCl.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	0.6	1.0	0.6	0.4	4.0	0.2	1.1
II.	22.0	57.6	66.0	47.2	70.0	66.2	54.8
III.	0.2	0.8	0.2	0.8	2.0	1.0	0.8
IV.	0.6	4.0	0.6	1.2	21.2	13.0	6.7
V.	0.6	0.4	0.6	0.2	0.6	...	0.5
	General average						12.8

TABLE XIV.

$\frac{N}{400}$ $C_2H_4O_2$.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	19.4	15.0	22.4	23.6	9.0	3.6	15.5
II.	62.4	78.8	75.6	55.0	29.6	71.6	62.1
III.	0.8	3.0	27.6	2.6	26.4	5.4	10.9
IV.	33.0	10.6	25.0	11.8	7.4	31.0	19.8
V.	20.8	1.0	8.4	5.4	0.8	...	7.3
	General average						23.1

TABLE XV.

$\frac{N}{400}$ HCl.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	0.4	0.4	0.4	0.4	0.2	0.4	0.4
II.	1.0	30.6	10.8	0.6	0.2	...	8.6
III.	0.6	0.4	21.4	0.4	1.0	19.4	7.2
IV.	0.4	1.0	11.0	28.4	...	...	10.2
V.	1.2	0.8	0.4	0.6	0.6	3.2	1.1
	General average						5.5

TABLE XVI.

$\frac{N}{400}$ $C_2H_4O_2$.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	4.0	1.0	5.2	5.0	1.6	57.8	12.4
II.	18.0	9.0	14.4	1.2	0.6	...	8.6
III.	44.4	16.8	22.0	2.0	...	29.8	23.0
IV.	1.4	17.2	5.6	...	...	...	8.0
V.	38.8	13.0	13.6	29.6	20.0	22.4	22.9
	General average						14.9

It will be seen by inspecting the tables that each solution was as a rule used on each one of the five worms six times. The worms were tested in such sequence that when the reaction-times for each acid were averaged the factor of exhaustion was so distributed as to be non-cumulative for any class.

Notwithstanding the care exercised in keeping the experimental conditions as nearly uniform as possible, it will be seen that the various worms present striking differences; some exhibiting characteristically slow, others quick reactions.

The slow reaction was in the main found associated with two modes of behavior, unlike each other. In not a few of the cases recorded, the portion of the worm anterior to the clitellum would rapidly contract as soon as it cut the surface of the solution, thus exposing a smaller receptive area to the action of the acid than would otherwise have been done. In such cases the worm was probably less stimulated and therefore would naturally be slower in withdrawing from the stimulating solution. In many of the slow reactions, the behavior was directly opposite of that just described. After cutting the surface of the solution, the anterior end of the worm would greatly elongate and squirm about, as if testing its environment in all directions. Such random movements executed in an endeavor to escape the stimulus are described by Jennings (:06) for the earthworm. A worm crawling upon a flat surface might escape an irritating stimulus with which it came in contact anteriorly, either

by forward movement in a direction different from that in which it had been moving, or by a backward contraction. When in a suspended condition, however, escape from a stimulating solution can be effected only by a contraction. It is highly probable, however, that the anterior elongations and squirming of the worm observed in some slow reactions corresponds to the effort of a crawling worm to escape an irritation by moving forward in a new direction. This ineffective forward reaction most likely delayed the backward one and so lengthened the reaction time. Although the quick reaction is the more characteristic one, the general average of slow and quick reactions may be taken as a fair measure of the reaction-time.

Assuming that these reaction-times are indicative of the degree of stimulation, it is clear from the figures in Tables I to XVI that hydrochloric acid is more stimulating than nitric, sulphuric, or acetic acids, but beyond this it is difficult to go. This method was therefore abandoned for one that gave through a greater number of worms a more immediate comparison of the different acids.

In this second set of observations, twenty-four instead of five worms were tested in all four acids and their relative reaction times were recorded in the manner previously described. The tests were so made that each acid had first place with some worm. Thus the possibility of error due to always passing from a stronger to a weaker acid with a given worm was eliminated. The results are recorded in the following table.

By inspecting this table, it will be seen that the order of acids arranged according to their stimulating value is hydrochloric, nitric, sulphuric, and acetic. Furthermore, that the reaction times for hydrochloric and nitric are practically identical, while that for sulphuric is nearly twice that for hydrochloric or nitric acids. The stimulating value of acetic acid, according to these results, is about half that of sulphuric and scarcely a third of that of hydrochloric and nitric acids.

These last conclusions differ from those of Kahlenberg ('98) for sensations of taste in man, in that at $\frac{N}{400}$ he was unable to distinguish between hydrochloric, nitric, and sulphuric acids. As Table XVII shows, the earthworm, on the contrary, distinguishes between these acids; and since in each solution the hydrogen ions are the stimulating elements, it must follow that the difference in reaction is due to a difference in concentration of these ions.

As compared with other acids, solutions of acetic acid are peculiar in that their sour taste is more intense than would be expected from their degree of dissociation. Kahlenberg, for instance, found that a $\frac{N}{200}$ solution of acetic acid, being dissociated only about 6 per cent, has

a sour taste about four times as strong as it would be expected to have, assuming that the taste is due simply to the hydrogen ions momentarily present. Richards obtained a similar result, in that he found that the acetic acid was about one-third as sour as an equivalent solution of hydrochloric acid, though the acetic acid was only dissociated to the extent of one-fourteenth as much as the hydrochloric acid was.

To determine whether this discrepancy existed also for the earthworm, the following test was made. Two solutions were prepared, an $\frac{N}{400}$ solution of acetic acid and a weaker solution of hydrochloric acid. Both solutions, however, contained equivalent numbers of hydrogen ions. Worms were now tested in these two solutions and the results are recorded in Tables XVIII and XIX.

It is clear from these tables that the earthworm, like man, is stimulated by acetic acid more vigorously than should be expected in accordance with the dissociation hypothesis. The explanation for this peculiarity cannot be given. It is not unlikely, however, that it may be ascribed to the undissociated molecules of acetic acid which in a way serve as a reverse for hydrogen ions.

TABLE XVII.

Reaction-Times, in Seconds, of Twenty-four Worms to Solutions of Hydrochloric, Nitric, Sulphuric, and Acetic Acids.

	No. of Worm.																								Average.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
HCl	.4	.6	51.0	.2	1.8	8.0	.4	.6	.4	.4	.2	19.6	.2	12.6	.2	.6	.2	.2	1.0	.2	.8	11.6	4.2	.4	4.7
HNO_3	.4	.4	32.4	.8	.4	11.4	. .	13.4	3.2	.4	.4	12.4	2.8	.2	.2	22.0	.2	.8	1.2	.4	.4	1.4	1.2	.2	4.8
H_2SO_4	20.4	.6	22.4	8.4	5.2	2.8	.2	15.4	7.0	.6	.4	. .	3.4	13.2	.6	19.0	.4	15.0	.6	.6	17.2	11.2	6.4	.4	7.5
$C_2H_4O_2$	30.0	18.0	18.2	20.4	21.0	8.0	19.2	31.2	7.4	2.2	5.4	18.6	16.8	. .	10.0	3.2	25.0	3.0	.8	14.0	16.0	25.0	13.6	.6	14.3

TABLE XVIII.

Reactions of Allolobophora foetida to Solutions of Hydrochloric and Acetic Acids containing Equivalent Numbers of Hydrogen ions.

$HCl \rightleftharpoons \frac{N}{400} C_2H_4O_2$.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	7.0	23.6	28.0	19.0	21.2	18.0	19.4
II.	17.2	15.0	19.0	18.0	8.8	18.2	16.0
III.	16.4	27.0	14.0	19.4	13.0	10.0	16.6
IV.	12.0	19.6	16.0	19.4	15.0	12.4	15.7
V.	12.6	11.2	11.0	7.4	16.6	13.0	11.9
	General average						15.9

TABLE XIX.

$\frac{N}{400} C_2H_4O_2$.

No. of Worm.	Trials.						Averages.
	1	2	3	4	5	6	
I.	23.2	17.0	27.0	11.0	18.2	15.0	18.5
II.	1.8	1.0	1.8	1.8	11.4	28.0	7.6
III.	16.2	6.2	9.0	10.0	17.0	19.4	12.9
IV.	5.6	7.6	4.4	19.4	13.0	15.0	10.8
V.	7.0	12.2	11.4	2.2	8.0	10.0	8.4
	General average						11.6

SUMMARY.

1. The responses of the earthworm Allolobophora foetida to solutions of acids may be ascribed to the hydrogen ions that they contain.

2. The reaction-time of the earthworm depends upon the number of hydrogen ions present in the solution of the acid.

3. Using the reaction-time as a basis, the earthworm was found to discriminate more certainly than man between solutions of acids at a concentration of $\frac{N}{400}$.

4. The response of the earthworm to solutions of acetic acid was more active than would have been anticipated from the degree of dissociation of this acid, and in this respect the earthworm's reactions are in agreement with human sensations as worked out by Kahlenberg, and by Richards.

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